

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
13 January 2005 (13.01.2005)

PCT

(10) International Publication Number
WO 2005/003170 A2

(51) International Patent Classification⁷: C07K 16/00

(21) International Application Number:
PCT/GB2004/002870

(22) International Filing Date: 1 July 2004 (01.07.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0315450.7 1 July 2003 (01.07.2003) GB

(71) Applicant (for all designated States except US): CELL-
TECH R & D LIMITED [GB/GB]; 208 Bath Road,
Slough, Berkshire SL1 3WE (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): HUMPHREYS,
David, Paul [GB/GB]; Celltech R & D Limited, 208 Bath
Road, Slough, Berkshire SL1 3WE (GB). HEYWOOD,
Sam, Philip [GB/GB]; Celltech R & D Limited, 208 Bath
Road, Slough, Berkshire SL1 3WE (GB). CARRING-
TON, Bruce [GB/GB]; Celltech R & D Limited, 208 Bath
Road, Slough, Berkshire SL1 3WE (GB).

(74) Agent: THOMPSON, John; Celltech R & D Limited, 208
Bath Road, Slough, Berkshire SL1 3WE (GB).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,
ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,
FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI,
SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished
upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: MODIFIED ANTIBODY FRAGMENTS

(57) **Abstract:** The present invention provides an antibody Fab or Fab' fragment to which at least one effector molecule is attached characterized in that the heavy chain in the fragment is not covalently bonded to the light chain and both the interchain cysteine of C_L and the interchain cysteine of C_H1 have been replaced with another amino acid.



WO 2005/003170 A2

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
13 January 2005 (13.01.2005)

PCT

(10) International Publication Number
WO 2005/003170 A3

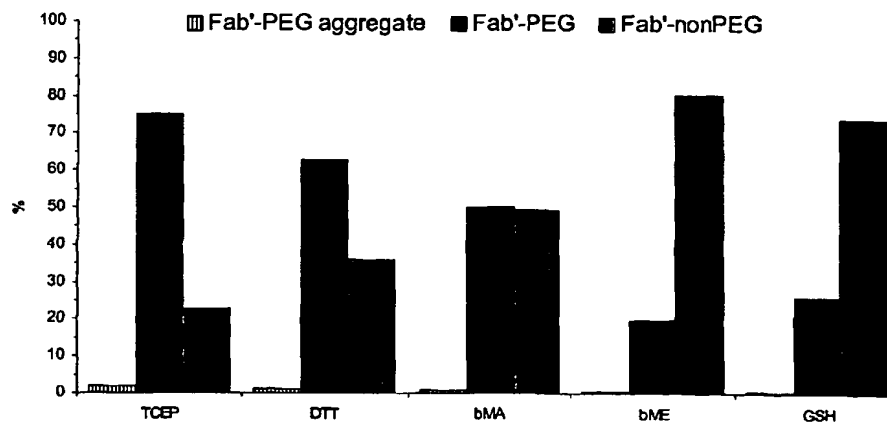
- (51) International Patent Classification⁷: **A61K 47/48**
- (21) International Application Number:
PCT/GB2004/002870
- (22) International Filing Date: 1 July 2004 (01.07.2004)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
0315450.7 1 July 2003 (01.07.2003) GB
- (71) Applicant (for all designated States except US): **CELL-TECH R & D LIMITED** [GB/GB]; 208 Bath Road, Slough, Berkshire SL1 3WE (GB).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **HUMPHREYS, David, Paul** [GB/GB]; Celltech R & D Limited, 208 Bath Road, Slough, Berkshire SL1 3WE (GB). **HEYWOOD, Sam, Philip** [GB/GB]; Celltech R & D Limited, 208 Bath Road, Slough, Berkshire SL1 3WE (GB). **CARRINGTON, Bruce** [GB/GB]; Celltech R & D Limited, 208 Bath Road, Slough, Berkshire SL1 3WE (GB).
- (74) Agent: **THOMPSON, John**; Celltech R & D Limited, 208 Bath Road, Slough, Berkshire SL1 3WE (GB).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

[Continued on next page]

(54) Title: MODIFIED ANTIBODY FRAGMENTS



(57) Abstract: The present invention provides an antibody Fab or Fab' fragment to which at least one effector molecule is attached characterized in that the heavy chain in the fragment is not covalently bonded to the light chain and both the interchain cysteine of C_L and the interchain cysteine of C_H1 have been replaced with another amino acid.

WO 2005/003170 A3



(88) Date of publication of the International search report:
10 March 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

PCT/GB2004/002870

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, PAJ, EMBASE, MEDLINE, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99/15549 A (CELLTECH THERAPEUTICS LTD ; HUMPHREYS DAVID PAUL (GB)) 1 April 1999 (1999-04-01)	1-19, 27-30
Y	page 24, line 8 - line 24; claims; examples; table 1	1-30
X	WO 97/36932 A (KALLAND TERJE ; PHARMACIA & UPJOHN AB (SE); ANTONSSON PER (SE); HANSSO) 9 October 1997 (1997-10-09)	1-7, 27, 28, 30
Y	page 24, line 18 - page 25, line 6; claims; examples	1-30
P,X	EP 1 419 786 A (BRACCO IMAGING SPA) 19 May 2004 (2004-05-19)	1,6-9,30
P,Y	page 4, line 33 - page 7, line 24; claims; examples	1-30
-/-		

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

G document member of the same patent family

Date of the actual completion of the international search

20 December 2004

Date of mailing of the international search report

04/01/2005

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Sommer, B

INTERNATIONAL SEARCH REPORT

PCT/GB2004/002870

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HUMPHREYS D P ET AL: "Formation of dimeric Fabs in Escherichia coli: effect of hinge size and isotype, presence of interchain disulphide bond, Fab' expression levels, tail piece sequences and growth conditions" JOURNAL OF IMMUNOLOGICAL METHODS, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL, vol. 209, no. 2, 1997, pages 193-202, XP004103797 ISSN: 0022-1759	1-15,17, 18,27, 28,30
Y	abstract; materials and methods; results; discussion;	1-30
X	HUMPHREYS D P ET AL: "Efficient site specific removal of a C-terminal FLAG fusion from a Fab' using copper(II) ion catalysed protein cleavage" PROTEIN ENGINEERING, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 12, no. 2, February 1999 (1999-02), pages 179-184, XP000882102 ISSN: 0269-2139	1-11,13, 15,17, 27,28,30
Y	abstract; materials and methods; results;	1-30
Y	BURMEISTER GETZ E ET AL: "Comparison between the sulfhydryl reductants tris(2-carboxyethyl)phosphine and dithiothreitol for use in protein biochemistry" ANALYTICAL BIOCHEMISTRY, ACADEMIC PRESS, SAN DIEGO, CA, US, vol. 273, 15 August 1999 (1999-08-15), pages 73-80, XP000946001 ISSN: 0003-2697 abstract; discussion;	19-26

INTERNATIONAL SEARCH REPORT

PCT/GB2004/002870

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
- a. type of material
- ☒ a sequence listing
- ☐ table(s) related to the sequence listing
- b. format of material
- ☒ in written format
- ☒ in computer readable form
- c. time of filing/furnishing
- ☐ contained in the international application as filed
- ☐ filed together with the international application in computer readable form
- ☒ furnished subsequently to this Authority for the purpose of search
2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

PCT/GB2004/002870

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9915549	A	01-04-1999	AT 257160 T AU 9174398 A DE 69820885 D1 DE 69820885 T2 EP 1015495 A2 ES 2212340 T3 WO 9915549 A2 JP 2001517423 T US 6642356 B1	15-01-2004 12-04-1999 05-02-2004 16-12-2004 05-07-2000 16-07-2004 01-04-1999 09-10-2001 04-11-2003
WO 9736932	A	09-10-1997	AU 707827 B2 AU 2525197 A BR 9702179 A CA 2222757 A1 CZ 9703712 A3 EP 0835266 A1 ID 16512 A JP 2002502363 T NO 975435 A NZ 329145 A PL 323626 A1 RU 2198895 C2 WO 9736932 A1 TW 517061 B US 2003092894 A1 US 6514498 B1 ZA 9702492 A	22-07-1999 22-10-1997 16-03-1999 09-10-1997 13-05-1998 15-04-1998 02-10-1997 22-01-2002 29-01-1998 23-06-2000 14-04-1998 20-02-2003 09-10-1997 11-01-2003 15-05-2003 04-02-2003 02-10-1997
EP 1419786	A	19-05-2004	EP 1419786 A1 WO 2004043492 A1	19-05-2004 27-05-2004